

Comparison of soymilk and probiotic soymilk effects on serum high-density lipoprotein cholesterol and low-density lipoprotein cholesterol in diabetic Wistar rats

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Original Article

Abstract

BACKGROUND: Soy milk (SM) and its fermented products are identified as rich sources of bioactive compounds helping to manage and to reduce the risk of chronic disease. This study aimed to compare the effects of SM and probiotic SM (PSM) consumption on serum low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) in diabetic Wistar rats.

METHODS: Probiotic SM was prepared by fermentation of the plain SM with a native strain of *Lactobacillus plantarum*. 20 streptozotocin-nicotinamide-induced diabetic Wistar rats were divided into two groups based on the type of administered SM (SM group and PSM group). The animals were fed with 1 ml/day of either soy or PSM for 21 days. The serum lipoprotein levels were analyzed at baseline and the end of the intervention period.

RESULTS: HDL-C increased significantly in PSM group. Furthermore, this group showed more percent of change in increased HDL-C in comparison with SM group ($P < 0.050$). Regarding LDL-C level, rats fed with SM was not significantly different from the PSM group ($P > 0.050$); though, this biomarker was reduced in both group.

CONCLUSION: Probiotic SM could modulate blood lipoprotein levels. Thus, it may be considered in managing diabetes complications and atherosclerotic risks.

Keywords: Lactobacillus, Probiotic, Low Density Lipoprotein Cholesterol, High Density Lipoprotein Cholesterol, Soy Milk

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Introduction

Diabetes is a chronic metabolic disease with growing prevalence and incidence throughout the world.¹ Apart from impaired glucose homeostasis, its complications usually occurred as a range of abnormalities in lipoprotein metabolism which results in dyslipidemia. Diabetic dyslipidemia is most commonly characterized by elevated levels of triglycerides, low levels of high-density lipoprotein cholesterol (HDL-C), and postprandial lipidemia. In addition, the low-density lipoprotein cholesterol (LDL-C) particles in diabetic dyslipidemia tend to convert a smaller and denser type which raises their atherogenicity.¹ The increasing rate of population suffered from diabetes (from 382 million to 592

million in 2035),² aging population, lack of sufficient physical activity, disobedience principles of appropriate nutrition,²⁻⁴ and an economic burden on the health care system⁵ highlights the necessity of doing more research on prevention and treatment of this disease.

Some bioactive ingredients in soy milk (SM) showed a protective effect against diabetes.^{6,7} Their intake through SM consumption was usually resulted in regulation of blood glucose and insulin levels and rising insulin sensitivity.^{8,9} On the other hand, SM fermentation increases the bioavailability of its isoflavones, as the beneficial effects of ultimate fermented product get higher than those of unfermented.¹⁰ Furthermore, soy proteins

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cooperation with soy isoflavones have demonstrated a positive role in reducing some atherogenic lipid and lipoproteins serum levels.¹¹ In addition, fermentation of SM probably associated with regulation of lipid metabolism genes expression.¹² From another point of view, fermented products with probiotic bacteria could decrement complications associated with diabetes.¹³⁻¹⁵ Probiotics are living microorganisms which modulate the specific function of an organism by activation of specific molecular pathways.¹⁶ Among various species of these microorganisms, *Lactobacillus* and *Bifidobacterium* genera are commonly used in the preparation of probiotic products.¹⁷ *Lactobacillus plantarum* A₇ (KC 355240, LA7) is a native strain with proven probiotic properties.¹⁸⁻²⁰ This strain of bacteria was found to be capable of decreasing serum total cholesterol, LDL-C and triglyceride in mice.¹⁹ The present study designed to evaluate the effects of probiotic SM (PSM) fermented by LA7 serum HDL-C and LDL-C in streptozotocin (STZ)-nicotinamide (NA)-induced diabetic rats.

Materials and Methods

In the present study, pasteurized plain SM was bought from Shir Soya Isfahan Company in Iran. Its composition and energy are shown in table 1. A strain of *L. plantarum* A₇ (KC 355240, LA7) which was obtained from microbial collection of food microbiology laboratory in School of Nutrition and Food Science, Isfahan University of Medical Sciences, was used for preparation PSM. Overnight cultures were provided with 1% inoculums in de-Mans–Rogosa broth (MRS) (Merck-Germany) and aerobically incubation at 37 °C for 24 h (Irankhodsaz-Iran). Active bacteria were obtained through 2 successive overnight cultures. Bacterial pellet was achieved by three steps centrifugation at 6000, 8000, 10,000 rpm each took 15 min (Seurita-B. Braun, Hamburg, Germany) following by washing with normal saline. Then suspension of bacterial prepared with 0.5 optical density at 620 (λ) wavelength determined by spectrophotometer (Jenway-UK). A flask containing SM (200 ml) was added with 1% (v/v) of the active culture, and the cultured samples were undergone fermentation at 37°C in an aerobic condition. Base of our study on pH and the growth of LA7 in SM revealed that cell numbers of LA7 can pick to 10⁹ colony form unit (CFU)/ml in the presence 9 h fermentation that followed in the preparation of PSM samples in the presented study (Figures 1 and 2).

Table 1. Nutrient composition and energy content of soy milk used in this study*

Content	Mean value
Fat (g/100g)	1.20
Protein (g/100g)	2.10
Carbohydrate (g/100g)	1.50
Energy (mJ/kg)	1.06

*Declared by producer: Shir Soya Isfahan Company

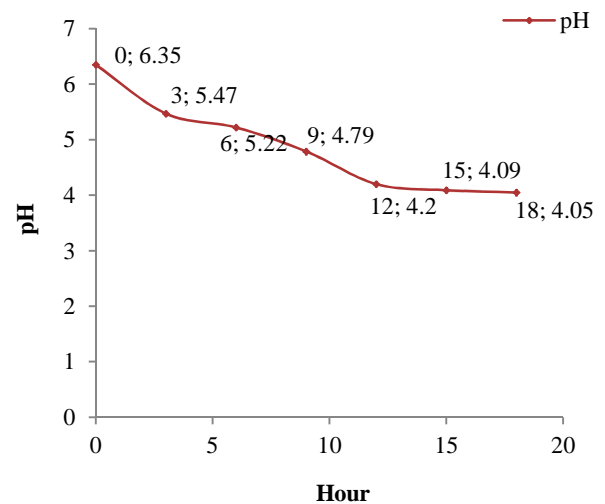


Figure 1. Variation curve of pH in fermentation time

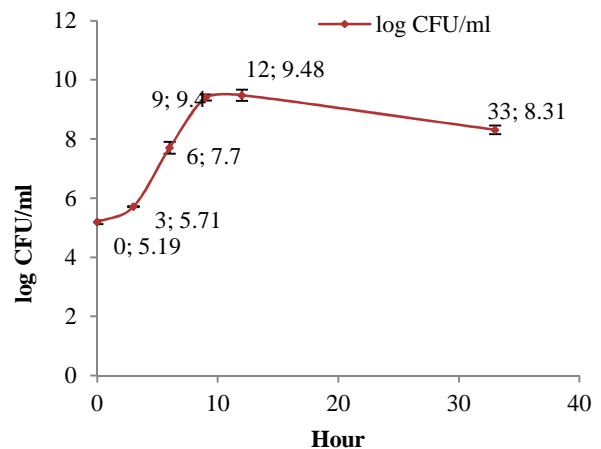


Figure 2. Growth curve of LA7 in fermentation time
CFU: Colony form unit

16-week-old male Wistar rats weighing about 185 ± 25 g which grew in the animal house of Isfahan School of Pharmacy and Pharmaceutical Sciences were housed in cages and subjected to a 12 h light/dark cycle with a maintained relative humidity of 45-55%, and temperature at 22 ± 2 °C. The animals were given free access standard pellet rat diet (Table 2) (Behparvar Company, Tehran, Iran) and water.

Table 2. The chemical composition and energy content of the standard pellet rat diet*

Contents	Value**
Protein (g/100 g)	19.50-20.5
Fat (g/100 g)	3.50-4.5
Fiber (g/100 g)	4.00-4.5
Ashe (g/100 g)	Maximum 10
Calcium (g/100 g)	0.95-1.0
Phosphorus (g/100 g)	0.65-0.7
Salt (g/100 g)	0.50-0.5
Moisture (g/100 g)	Maximum 10
Lysine (g/100 g)	1.15
Methionine (g/100 g)	0.33
Threonine (g/100 g)	0.72
Tryptophan (g/100 g)	0.25
Energy (MJ/kg)	16.16-17.0

* Declared by producer: Behparvar Company, Tehran, Iran;

** Data presented in ranges show minimum and maximum values

Diabetes was induced in overnight fasted rats by administering a single intraperitoneal injection of freshly prepared STZ (Sigma-Aldrich Company, Germany) 50 mg/kg b.w. followed 15 min later by 100 mg/kg of NA (Sigma-Aldrich Company, Germany) in 0.1 M citrate buffer (pH = 4.5).²¹⁻²⁴ Diabetes was confirmed in the STZ-NA-treated rats by measuring fasting blood glucose levels after one week of induction. Rats with fasting blood glucose of more than 200 mg/dl were considered as diabetics.²⁴ Then they were randomly divided into two separate groups of SM and (PSM) PSM groups of 10 rats in each.

The study was carried out according to the guidelines of Research Ethics Committee for animal experiments set forth by Isfahan University of Medical Sciences.

During the study period, the SM and PSM groups of rats were gavaged 1 ml/day by plain SM and PSM samples, respectively. Blood samples were collected by microcapillary tube from orbital sinus plexus under light ether anesthesia²⁵ after overnight starvation at 1st day and 21st day of the study. For analysis of blood lipoprotein, serum was obtained by centrifugation at 3000 rpm for 20 min and stored in a freezer at -80 °C until analysis. HDL-C and LDL-C concentrations were determined by using commercial kit (Biosystems, Spain) on A₁₅-Autoanalyzer set (Biosystems, Barcelona, Spain).

The data were expressed as mean \pm standard error. Values were evaluated by IBM SPSS for Windows (version 20, SPSS Inc., Chicago, IL, USA). The percent change for each variable was also obtained by the formula $(E-B)/B \times 100$, where E is the end-of-treatment values and B is the baseline values. The values of HDL-C and LDL-C in the two groups were compared using independent sample t-tests. The changes in the level of variables between the beginning and end of the intervention were compared by paired sample t-test. Differences with $P < 0.050$ were considered to be statistically significant.

Results

Serum HDL-C levels of PSM group were significantly increased in comparison with the relevant levels in SM group at the end of the intervention period ($P = 0.013$) (Table 3). Furthermore, at the end of the study, PSM group demonstrated a significant increase in serum HDL-C levels compared to the values in the baseline ($P = 0.044$) (Table 3). Moreover, in this group, more percent change in serum HDL-C level was calculated than that of SM group (16.8 vs. 9.036%) (Table 3).

Table 3. Serum levels of low-density lipoprotein cholesterol (LDL-C) and High-density lipoprotein cholesterol (HDL-C) in the study groups

Variable	Soy milk	Probiotic soy milk	P*
HDL-C (mg/dl)			
Baseline	30.71 \pm 3.33	32.66 \pm 2.44	0.608
After intervention	32.14 \pm 1.89	39.48 \pm 2.14	0.013
Percent change	9.36 \pm 7.70	16.80 \pm 5.10	0.491
P**	0.693	0.044	
LDL-C (mg/dl)			
Baseline	9.33 \pm 1.65	15.33 \pm 3.96	0.143
After intervention	7.10 \pm 0.63	10.01 \pm 4.48	0.407
Percent change	3.30 \pm 20.77	-13.31 \pm 19	0.688
P**	0.159	0.376	

Values are presented as mean \pm standard error; * P values refer to mean comparisons between groups (independent t-test); ** P values refer to variation from day 0 to day 21 within groups (paired t-test); LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol

LDL-C levels in both groups of rats were relatively decreased through the study period. This reduction was not sufficient to meet the statistical difference neither between the groups ($P = 0.407$) nor among each of the SM group ($P = 0.159$) and PSM group ($P = 0.376$). However, LDL-C percent change in PSM group were more than SM group (13.31 vs. 3.3%, $P = 0.688$) (Table 3).

Discussion

In this study, although serum LDL-C concentration in both treated groups of rats was not significantly reduced, but in PSM group, administration of fermented product led to more change in serum LDL-C concentration. PSM group, also, developed more percent change in increased HDL-C level than SM group.

The results of present study were in accordance with the results of those studies that claimed on SM inhibition effects against diabetes progression and its complications exacerbation through glycemic control.²⁶⁻²⁸

According to some previous studies, SM isoflavones enhance lipoprotein clearance and HDL-C biogenesis through the regulation of gene expression related to nuclear receptors of peroxisome proliferator-activated receptor and can improve glucose and lipid metabolism.^{12,29-32} Furthermore, they regulate beta-cell secretory pattern partially by activating the signal transduction pathway, CAMP/protein kinase.³³ In addition, soy peptides may lead to inducing the expression of LDL-C receptors in liver and decreased the levels of serum cholesterol.³⁴

Fermentation process led to structural and functional changes in soy bean which provided the production of a diverse mix of peptides and amino acids with advantageous physiological effects.³⁵⁻³⁷ Furthermore, fermentation converts glucoside isoflavones to aglycone one that has higher physiological activity.³⁸ Inhibitory activity against the enzyme glucosidase and alpha-amylase in fermented SM is more than SM.⁸

Xie et al. Showed that supplementation of *L. plantarum* 9-41-A and *Lactobacillus fermentum* M1-16 in rats under high cholesterol diet a 6 week feeding period impose beneficial effects in triglycerides, total cholesterol, and LDL-C which are associated with positive impacts on intestinal *Lactobacillus* and *Bifidobacterium* bacteria population as well as more body weight lost.³⁹ In that study, *L. plantarum* 9-41-A was more effective than *L. fermentum* M1-16 in above-mentioned

effects, since; authors proposed that *L. plantarum* may be involved in lipid metabolism.

Results of this study are consistent with the results of Rossi et al. In that intake of the soy product fermented by *Enterococcus faecium* and *Lactobacillus jugurti* cause a 17.8% increase in the level of the HDL-C fraction in hypercholesterolemic rabbits during 30 day.³⁵ Though, in the present study less percent change in HDL-C enhancement, about 7.8% was resulted that might be due to shorter study period than the above-mentioned study.

Wang et al. demonstrated that 4 weeks consumption of 2 ml of fermented SM by *L. plantarum* P-8 in experimental hyperlipidemic rats caused significant changes in the serum LDL-C and HDL-C comparing the control group having only physiological slain but not in the group of rat that was fed with plain SM. In that study, a notable decrease in LDL-C serum and an increment of HDL-C were observed in the rats treated with fermented soy compared with control group. These changes only relatively were occurred in the plain soy group. Furthermore, fermented SM enhanced total bile acids and lipid levels in fecal hyperlipidemic rats.⁴⁰ In comparison to our results, the trends in LDL-C reduction and HDL-C increase are comparable to the latter study, however, in the present study, HDL-C enhancement by fermented SM was definitely shown to be more effective than the unfermented relevant product.

It can be concluded that PSM could be more effective in increasing HDL-C level and decreasing LDL-C level than SM.

Development of human studies on PSM is suggested in the future that could improve the finding of this study. Furthermore, formulation of PSM for human consumption considering the society's attitudes toward drinkable SM can be considered in future studies.

A noticeable difference in serum LDL-C levels (9.33 vs. 15.33) between compared groups in the baseline could be regarded as a limitation of this study which may interfere with the obtained results on serum LDL-C. Characterization of serum LDL-C and HDL-C in the tested rats before their grouping would have rectified such a source of error. Nevertheless, this may restrain the sampling randomization.

Conclusion

Probiotic SM by LA7 could provide higher HDL-C levels and lower LDL-C levels in comparison with

plain SM. It seems PSM may be more effective in controlling diabetes and reduction its complications associated with atherosclerosis.

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Conflict of Interests

Authors have no conflict of interests.

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